THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Previously presented) A method for amplifying a signal using a flow cytometer for detection of a polynucleotide, comprising the steps of

(a) providing at least one identifiable, fluorescent detectable microsphere coupled with at least one pre-optimized oligonucleotide;

(b) hybridizing a labeled target polynucleotide to said oligonucleotide to form an oligonucleotide/target polynucleotide complex, wherein said complex comprises a detectable signal through the binding of a receptor to the label;

(c) providing a labeled ligand for said receptor, wherein when said ligand binds said receptor, said signal is amplified; and

(d) identifying said microsphere and quantifying the amplified labeled ligand signal via flow cytometry.

2. (Original) The method of claim 1, wherein the pre-optimized oligonucleotide is selected with an algorithm.

3. (Original) The method of claim 2, wherein said algorithm utilizes at least one of the following selection criteria:

(a) selecting at least one perfect match pre-optimized oligonucleotide, wherein the selected at least one perfect match pre-optimized oligonucleotide has an acceptable measure of correlation with a standard gene expression value;

(b) selecting at least one perfect match and minus mismatch pre-optimized oligonucleotide pair, wherein within a pair the selected at least one perfect match pre-optimized oligonucleotide minus the mismatch pre-optimized oligonucleotide has an acceptable measure of correlation with a standard gene expression value;

(c) selecting at least one pair of pre-optimized oligonucleotides from different pre-optimized oligonucleotide sets, wherein the ratio of signals in the pre-optimized of igonucleotides in the at least one pair of pre-optimized oligonucleotides has an

acceptable correlation with a standard signal ratio; and

(d) selecting at least one perfect match pre-optimized oligonucleotide, wherein the perfect match pre-optimized oligonucleotide has an acceptable relative standard deviation.

4. (Original) The method of claim 1, wherein said pre-optimized oligonucleotide is further defined as being selected by the steps of:

providing a sample comprising at least one target polynucleotide;

subjecting said sample to an array of oligonucleotides, wherein the hybridization of said target polynucleotide to at least one oligonucleotide in the array provides a detectable hybridization fingerprint; and

identifying at least one optimal oligonucleotide from said fingerprint.

5. (Original) The method of claim 1, wherein said pre-optimizcd oligonucleotide is further defined as being selected by the steps of:

providing a sample comprising a plurality of target polynucleotides, said target polynucleotides defined as RNA polynuclemides from more than one gene;

subjecting said sample to an array of oligonucleotides, wherein the hybridization of more than one different RNA polynucleotide to a respective oligonucleotide in the array provides a detectable hybridization fingerprint for more than one gene; and

identifying at least one optimal oligonucleotide for said more than one gene from said fingerprint.

6. (Original) The method of claim 1, wherein said identifying step utilizes an algorithm to identify said oligonucleotide.

7. (Original) The method of claim 6, wherein said algorithm identifies an oligonucleotide having complete complementarity to at least a portion of said target polynucleotide.

8. (Original) The method of claim 1, wherein the target polynucleotide is comprised in a plurality of RNA polynucleotides and the concentration of said plurality is from about 1 μg to about 10 μg.

9. (Original) The method of claim 1, wherein said ligand comprises an antibody.

10. (Original) The method of claim 1, wherein the label of the target polynucleotide and/or the label of the ligand comprises a fluorescent label, an enzyme label, a chemical label, or a gold label.

11. (Original) The method of claim 1, wherein the label of the target polynucleotide and the label of the ligand are identical.

12. (Previously presented) The method of claim 1, wherein said identifiable, fluorescent detectable microsphere is comprised in a plurality of identifiable, fluorescent detectable microspheres and said target polynucleotide is comprised in a plurality of RNA polynucleotides.

13. (Original) The method of claim 12, wherein the plurality of RNA polynucleotides is comprised in a mRNA-containing sample, and said method is further defined as a method for providing mRNA expression profiling information.

14. (Original) The method of claim 12, wherein at least one microsphere in said plurality of microspheres comprises different oligonucleotides from the oligonucleotides of another microsphere in said plurality.

15. (Previously presented) The method of claim 12, wherein at least one identifiable fluorescent detectable microsphere in the plurality comprises more than one nonidentical pre-optimized oligonucleotide having sequence complementary to the same RNA polynucleotide.

16. (Currently amended) A composition, comprising:

a plurality of identifiable, fluorescent detectable microspheres, each identifiable fluorescent detectable microsphere linked to at least one pre-optimized oligonucleotide, wherein said oligonucleotide is hybridized to a labeled RNA polynucleotide forming an oligonucleotide/labeled RNA polynucleotide hybridized complex, and wherein said complex comprises a detectable signal through the binding of a receptor to the label, said signal amplified upon binding of a labeled ligand for the receptor~~; and identifying said micropsheres and quantifying the amplified labeled ligand signal via flow cytometry~~.

17. (Previously presented) The composition of claim 16, wherein at least one

identifiable, fluorescent detectable microsphere in said plurality of identifiable, fluorescent detectable microspheres comprises different oligonucleotides from the oligonucleotides of another identifiable, fluorescent detectable microsphere in said plurality.

18. (Previously presented) The composition of claim 16, wherein at least one identifiable, fluorescent detectable microsphere in the plurality comprises more than one non-identical pre-optimized oligonucleotide each having sequence complementary to the same RNA pigynucleotide.

19. (Withdrawn) A method of optimizing an oligonucleotide hybridization-based assay, comprising the steps of:

providing a sample comprising at least one target polynucleotide;

subjecting said sample to an array of oligonucleotides, wherein the hybridization of said target polynucleotide to at least one oligonucleotide in the array provides a detectable hybridization fingerprint;

identifying at least one optimal oligonucleotide from said fingerprint, wherein said identifying step utilizes an algorithm defined by at least one of the following selection criteria:

(a) selecting at least one perfect match pre-optimized oliganuclemide, wherein the selected at least one perfect match pre-optimized oligonucleotide has an acceptable measure of correlation with a standard gene expression value;

(b) selecting at least one perfect match and minus mismatch pre-optimized oligonucleotide pair, wherein within a pair the selected at least one perfect match pre-optimized oligonucleotide minus the mismatch pre-optimized oligonucleotide has an acceptable measure of correlation with a standard gene expression value',

(c) selecting at least one pair of pre-optimized oligonucleotides from different pre-optimized oligonucleotide sets, wherein the ratio of signals in the pre-optimized oligonucleotides in the at least one pair of pre-optimized

oligonucleotides has an acceptable correlation with a standard signal ratio; and

(d) selecting at least one perfect match pre-optimized oligonucleotide, wherein the perfect match pre-optimized oligonucleotide has an acceptable relative standard deviation; and

subjecting said optimal oligonucleotide to an oligonucleotide hybridization-based assay.